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(54) Title: A PROCESS FOR THE PREPARATION OF ENANTIOMERIC 2-ALKANOIC ACIDS

(57) Abstract

This invention relates to the enantioselective biologically-catalyzed hydrolysis of certain racemic nitriles to the correspond-
ing R- or S-amides, chemically or biologically-catalyzed hydrolysis of the amides to the corresponding R- or S-acids in a batch
process or in a continuous process that employs racemization and recycling of enantiomeric nitrile intermediates, the racemic ni-
triles being selected from the group, A-C(R¹)(R²)CN, wherein A, R¹ and R² are as defined in the text, as well as certain bio-
logical materials employed to catalyze the process.

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TITLEA PROCESS FOR THE PREPARATION OF
ENANTIOMERIC 2-ALKANOIC ACIDSFIELD OF THE INVENTION

10 Enantioselective biologically-catalyzed hydrolysis of nitriles to the corresponding enantiomers of 2-alkanoic acids via enantiomeric amide intermediates.

STATE OF THE ART

15 Many agrichemicals and pharmaceuticals of the general formula, X-CHR-COOH, are currently marketed as racemic or diastereomeric mixtures. In many cases, the physiological effect derives from only one enantiomer/diastereomer while the other enantiomer/diastereomer is inactive or even harmful. Chemical and enzymatic techniques for separating enantiomers are becoming increasingly important tools for
20 production of chemicals of high enantiomer purity.

WO 86/07386 discloses a process for preparing amino acids or amino acid amides from an enantiomeric mixture of the corresponding amino nitrile with an enantioselective nitrilase and subsequent recovery of the resulting optically-active amino acid or amino amide. This
25 publication does not suggest the instant invention because it utilizes different microorganisms and the hydrolyses described are stereoselective, not stereospecific.

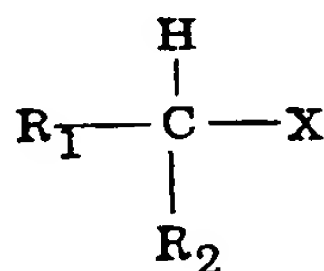
EPA 326,482 discloses the stereospecific preparation of aryl-2-alkanoic acids such as 2-(4-chlorophenyl)-3-methylbutyric acid by
30 microbial hydrolysis of the corresponding racemic amide. Microorganisms disclosed in EPA 326,482 include members of Brevibacterium and Corynebacterium. The process was performed batchwise without organic solvent, and the enzymatically-active material was discarded after being used once. Data in the examples of EPA
35 326,482 indicate that 35 to 60% of the S-amide remained unconverted. The enantiomeric excess of the S-acid produced was 92 to 97%.

5 U.S. 4,366,250 discloses a process for preparing L-amino acids from the corresponding racemic amino nitrile with bacteria having a general nitrile hydratase and a L-stereospecific amidase. Microorganisms are chosen from Bacillus, Bacteridium, Micrococcus and Brevibacterium.

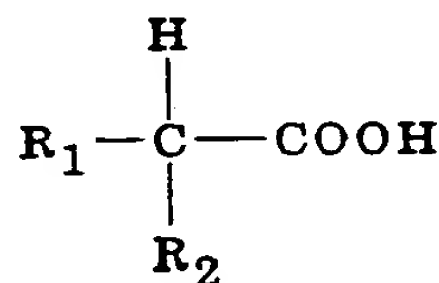
10 EPA 356,912 discloses preparation of optically-active 2-substituted carboxylic acids by hydrolysis of the corresponding racemic nitrile in the presence of a microorganism or enzyme. The microorganisms employed do not suggest those found herein to convert nitriles to the amide precursors of the acids.

15 EPA 348,901 discloses a process for producing an optically-active α -substituted organic acid of Formula ii by treating a racemic α -substituted nitrile or amide of Formula i with a microorganism selected from the group Alcaligenes, Pseudomonas, Rhodopseudomonas, Corynebacterium, Acinetobacter, Bacillus, Mycobacterium, Rhodococcus and Candida;

20



i



ii

wherein:

25 R_1 and R_2 each represent halogen; hydroxy; substituted or unsubstituted alkyl, cycloalkyl, alkoxy, aryl, aryloxy or heterocycle; provided that R_1 and R_2 are different; and X is a nitrile or amido group. See also, Yamamoto et al., Appl. Envir. Microbiol., 56(10), 3125-9, 1990.

30

EPA 330,529 discloses a process employing Brevibacterium and Corynebacterium for the preparation of the S-enantiomers of aryl-2-propionic acids of Formula iii



iii

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from the corresponding racemic aryl-2-propionamide wherein Ar represents a substituted or unsubstituted monocyclic or polycyclic aromatic or heteroaromatic radical.

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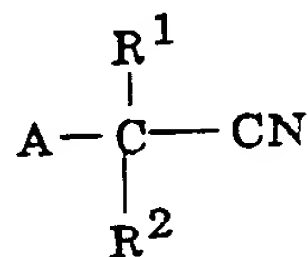
U.S. 4,800,162 discloses the resolution of racemic mixtures of optically-active compounds such as esters, amides, carboxylic acids, alcohols and amines using multiphase and extractive enzyme membranes.

15

SUMMARY OF THE INVENTION

This invention concerns certain individual and combined steps in a biologically-catalyzed method for converting a racemic alkyl nitrile to the corresponding R- or S-alkanoic acid through an intermediate amide. The starting nitrile is:

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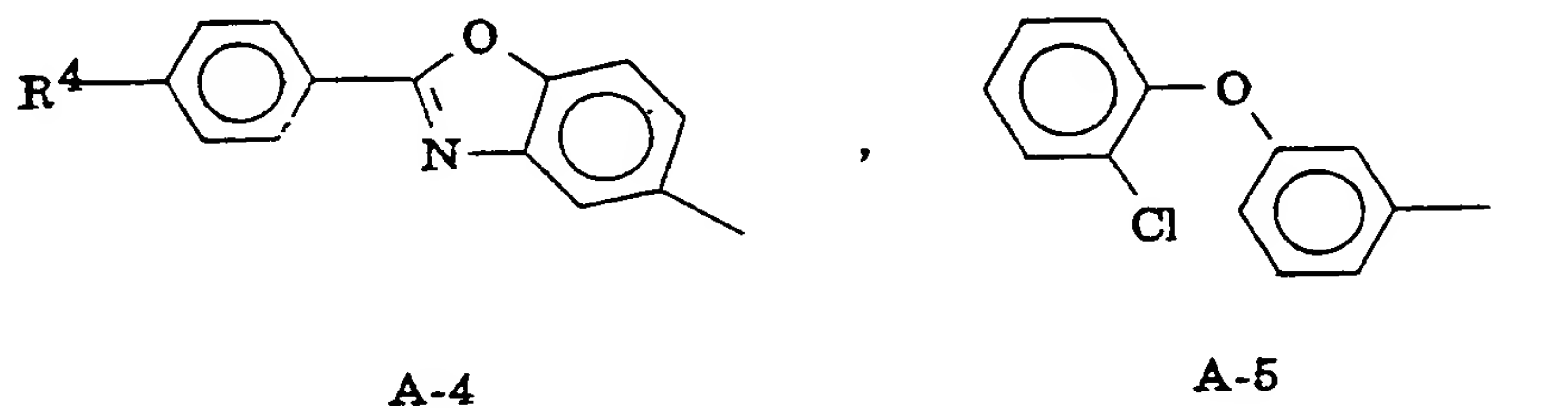
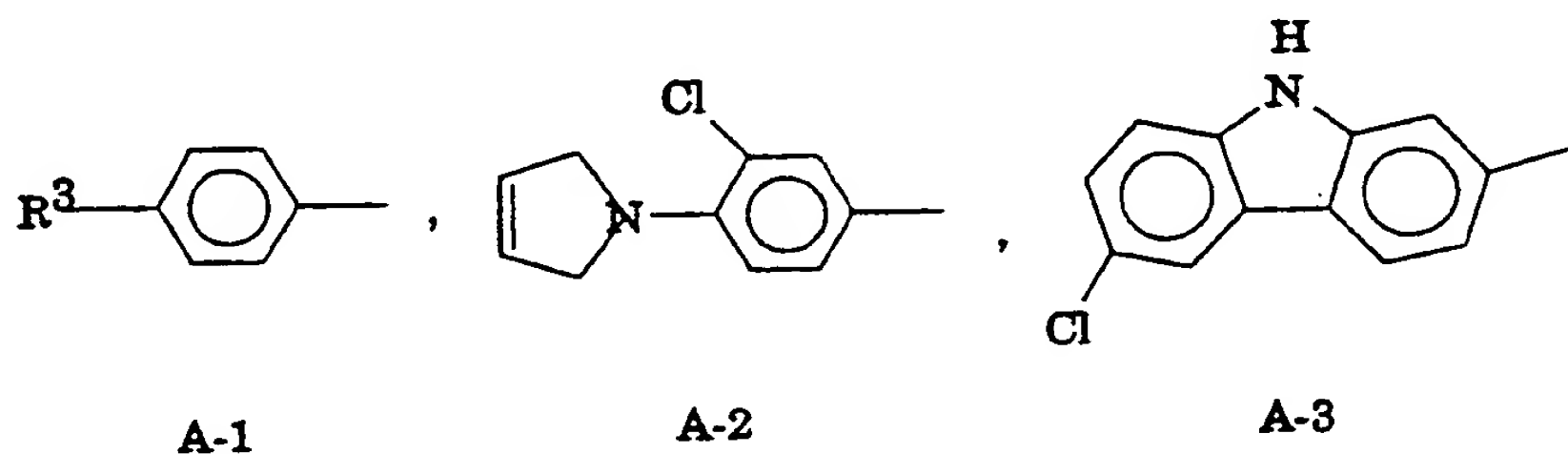


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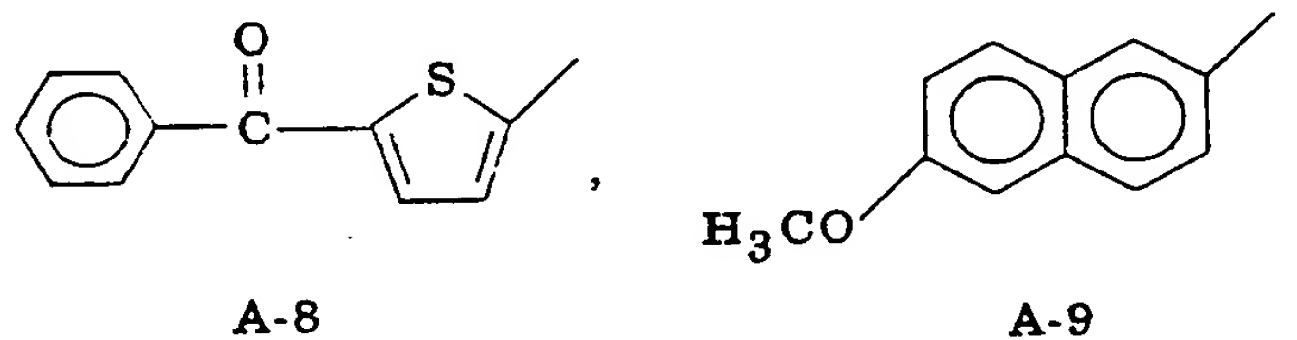
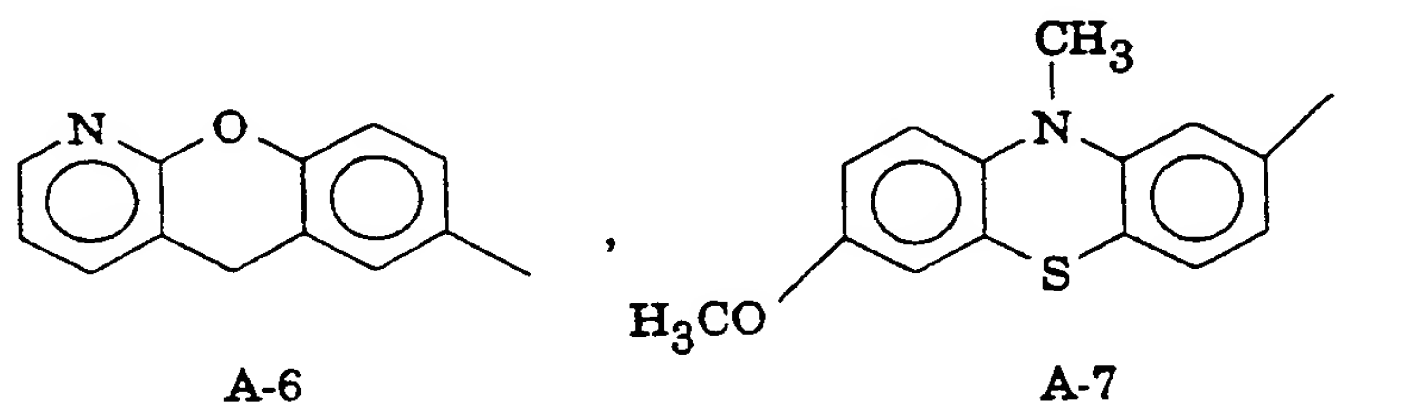
(R,S)

5 wherein:

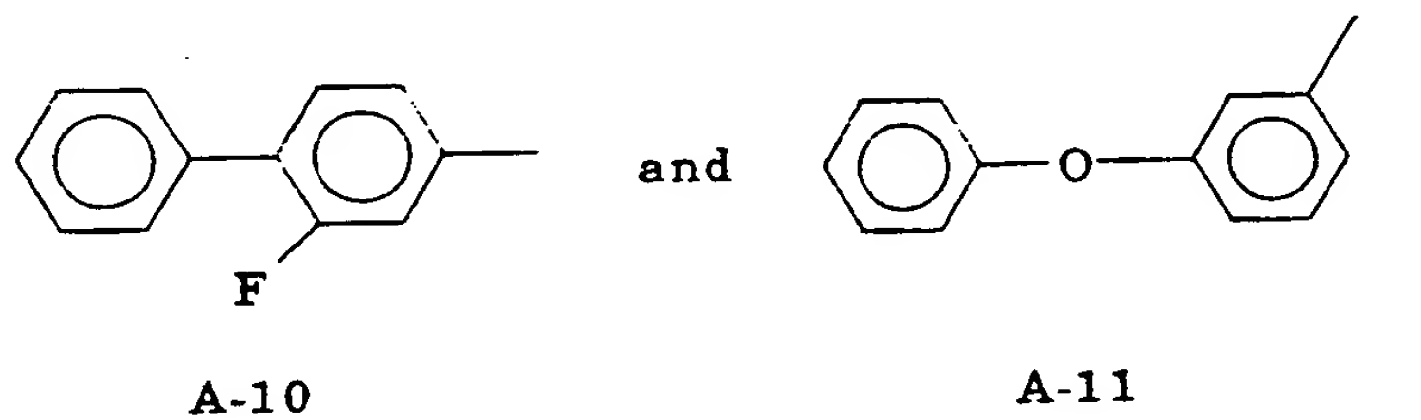
A is selected from the group consisting of:



10



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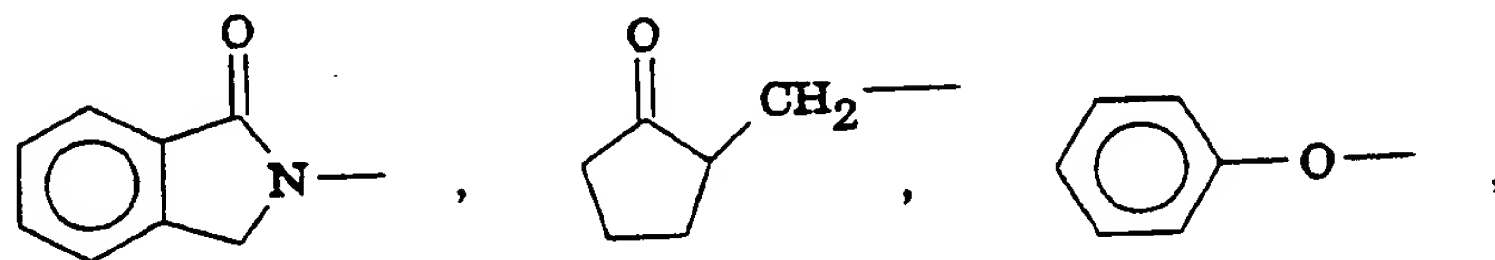


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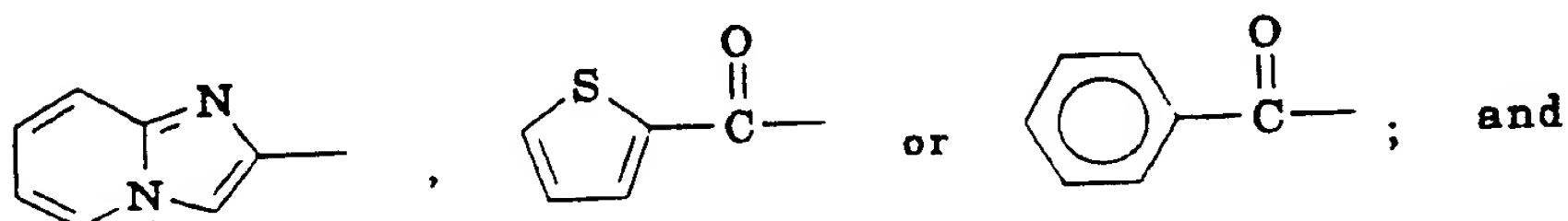
R^1 is C_1 - C_4 alkyl;

R^2 is H or OH;

R^3 is H, Cl, OCF_2H , $(CH_3)_2CHCH_2$, $H_2C=C(CH_3)CH_2NH$,

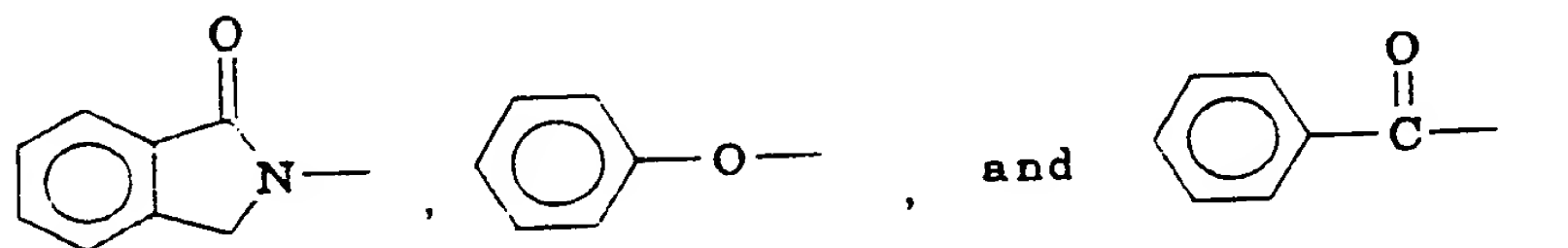


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R^4 is Cl or F.

15 Preferred values of A are A-1, A-5, A-9, A-10 and A-11. Preferred values of A-1 are those wherein R^3 is selected from the group Cl, $(CH_3)_2CHCH_2$,



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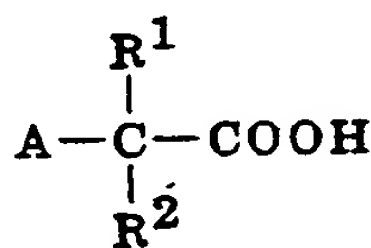
Preferred values for R^1 are CH_3 and $CH(CH_3)_2$.

Preparation of the amide, in Step i of the method of this invention, comprises contacting I with a biological material that stereospecifically converts the R,S mixture of nitriles of Formula I to either the R- or S- amide wherein said R- or S-amide is substantially free of the opposite enantiomer. Resolution of the mixed R- and S-enantiomer of a nitrile of Formula I to resolved amide is followed by conversion to the corresponding acid of Formula II by Step ii of the method of this invention:

25

30

5



II

10 The amide intermediate is A-C(R¹)(R²)-CONH₂. This invention also concerns the racemization (Step iii) and subsequent recycle of unconverted R- or S-nitrile, when R² is H, from Step ii back to the original reactor in a continuous process. In the continuous process, the racemic alkyl nitrile starting reactant is contacted with biological material containing or comprising nitrile hydratase and amidase enzymes at the same time or consecutively to proceed first to the amide (Step i) and then to the acid (Step ii). Alkyl acid is continually removed and by-product R- or S-alkyl nitrile in which R² is H is racemized and recycled in a continuous process in which it is combined with additional alkyl nitrile and contacted with enzyme(s) to form the alkyl amide and then the acid.

20 This invention is particularly characterized by the biological material (a microorganism or variant or mutant thereof, or an enzyme) employed in Step i and by the combination of biological catalysis (Step i) with mineral acid hydrolysis (Step ii) or known amidase enzymes (Step ii). The nitrile racemization is characterized by the use of a strongly basic ion exchange resin in the absence of any substantial amount of water and most preferably in the presence of a nonaqueous solvent such as methanol, ethanol, toluene, dioxane and the like. To simplify the description of this invention, the method will be explained with reference to the enzymes found to be useful.

30 Preferred Step i enzymes comprise those found in the following microorganisms: Pseudomonas spp., e.g., putida, aureofaciens, Moraxella spp., Serratia, e.g., Serratia liquefaciens. These enzymes can be isolated or biosynthesized and used as such but it is usually more convenient to employ the appropriate microorganism(s).

5 In this method for hydrating and converting an R,S mixture of nitrile to the corresponding R- or S- enantiomeric acid, Step i is accomplished by the action of a stereospecific nitrile hydratase enzyme originating in a microorganism which is obtained by culturing the microorganism in the presence of a medium suitable for production of the stereospecific nitrile hydratase. This medium may include nitriles or
10 amides as enzyme inducers or in the case of Pseudomonas putida 5B-MGN-2p, which produces the enzyme constitutively in the absence of an inducer, need include only an appropriate source of nitrogen for growth (e.g., ammonium chloride). The nitrile hydratase thus obtained is
15 added to act upon either R- or S-nitriles to yield the corresponding R- or S-amides. In Step ii, the R- or S-amide is hydrolyzed by mineral acid or amidase enzyme to the corresponding R- or S-acid.

This two-step method results in a mixture of an R- or S-acid and an S- or R-nitrile, respectively. Chiral nitrile and acid are first separated
20 by neutralization and solvent extraction. Then, the chiral nitrile is racemized into a mixture of R,S nitrile which can again be hydrolyzed stereospecifically into R- or S-amide by the action of the stereospecific nitrile hydratase described in Step i.

One method for inducing the nitrile hydratase to act upon the
25 nitrile is to collect the enzyme from the microorganism that produces it and use the enzyme as an enzyme preparation in a biologically-recognized manner.

This invention also concerns a biological material located in or derived from Pseudomonas sp. 3L-G-1-5-1a, Pseudomonas sp. 2G-8-5-1a,
30 P. putida 5B-MGN-2p and P. aureofaciens MOB C2-1, or a variant or mutant thereof, which material stereospecifically converts a racemic nitrile to the corresponding enantiomeric R- or S-amide.

DETAILS OF THE INVENTION

In the context of the present disclosure, the terms "stereospecific
35 reaction" or "stereospecific nitrile hydratase" are defined by the enantiomeric ratio (E) for the R- and S-enantiomers. E corresponds

5 to the ratio of the rate of reaction of the two enantiomers. When E is high, i.e., greater than 7, the reaction is stereospecific and when E is less than 7, the reaction is stereoselective. Preferred reactions are those wherein E is above 8.5 and most preferred reactions are those wherein E is 10 or above.

10 Abbreviations

	CPIN	-	2-(4-chlorophenyl)-3-methylbutyronitrile
	CPIAm	-	2-(4-chlorophenyl)-3-methylbutyramide
	CPIA	-	2-(4-chlorophenyl)-3-methylbutyric acid
	IBCN	-	2-(4-isobutylphenyl)-propionitrile
15	IBAm	-	2-(4-isobutylphenyl)-propionamide
	IBAC	-	2-(4-isobutylphenyl)-propionic acid (ibuprofen)
	NPCN	-	2-(6-methoxy-2-naphthyl)-propionitrile
	NPAAm	-	2-(6-methoxy-2-naphthyl)-propionamide
	NPAC	-	2-(6-methoxy-2-naphthyl)-propionic acid
20	HPLC	-	High-Performance Liquid Chromatography
	GC	-	Gas Chromatography
	DMSO	-	Dimethylsulfoxide.

Step i

25 The microorganisms used in the present invention belong to the genera Pseudomonas, Moraxella, and Serratia. Representative strains include P. putida, 5B-MGN-2P; Moraxella sp., 3L-A-1-5-1a-1; P. putida, 13-5S-ACN-2a; Pseudomonas sp., 3L-G-1-5-1a; and Serratia liquefaciens, MOB IM/N3. These strains were deposited under the terms of the
30 Budapest Treaty at NRRL (Northern Regional Research Center, U.S. Department of Agriculture, 1815 North University St., Peoria, IL) and bear the following accession numbers:

P. putida 5B-MGN-2P, NRRL-B-18668
Moraxella sp. 3L-A-1-5-1a-1, NRRL-B-18671
35 P. putida 13-5S-ACN-2a, NRRL-B-18669
Pseudomonas sp. 3L-G-1-5-1a, NRRL-B-18670
Serratia liquefaciens, MOB IM/N3, NRRL-B-18821

- 5 P. putida 2D-11-5-1b, NRRL-B-18820
 Pseudomonas sp. 2D-11-5-1c, NRRL-B-18819
 Pseudomonas sp. 2G-8-5-1a, NRRL-B-18833
 P. aureofaciens, MOB C2-1, NRRL-B-18834.

The above strains were isolated from soil collected in Orange, Texas.

- 10 Standard enrichment procedures were used with the following modified medium (PR Basal Medium).

PR Basal Medium

		<u>g/L</u>
15	KH ₂ PO ₄	8.85
	Sodium citrate	0.225
	MgSO ₄ •7H ₂ O	0.5
	FeSO ₄ •7H ₂ O	0.05
	FeCl ₂ •4H ₂ O	0.0015
20	CoCl ₂ •6H ₂ O	0.0002
	MnCl ₂ •4H ₂ O	0.0001
	ZnCl ₂	0.00007
	H ₃ BO ₃	0.000062
	NaMoO ₄ •2H ₂ O	0.000036
25	NiCl ₂ •6H ₂ O	0.000024
	CuCl ₂ •2H ₂ O	0.000017
	Biotin	0.00001
	Folic acid	0.00005
	Pyridoxine•HCl	0.000025
30	Riboflavin	0.000025
	Nicotinic acid	0.000025
	Pantothenic acid	0.00025
	Vitamin B ₁₂	0.000007
	P-Aminobenzoic acid	0.00025
35	The following additions and or modifications were made to the PR basal medium for enrichments described above:	

Strain	Enrichment Nitrile (25 mM)	pH	Other
5B-MGN-2P	(±)-2-methylglutaronitrile (Aldrich Chem. Co., Milwaukee, WI)	7.2	30 disodium succinate/L
3L-A-1-5-1a-1	(±)-2-methylglutaronitrile	5.6	30 g glucose/L
3L-G-1-5-1a			
13-5S-ACN-2a	acetonitrile (Aldrich Chem. Co., Milwaukee, WI)	7.2	30 g disodium succinate/L

5

Strains were initially selected based on growth and ammonia production on the enrichment nitrile. Isolates were purified by repeated passing on Bacto Brain Heart Infusion Agar followed by screening for ammonia production from the enrichment nitrile.

10

Purified strains were identified based on membrane fatty acid analysis of the methyl esters following standard protocols (Mukawaya et al., J. Clin. Microbial, 1989, 27:2640-46) using the Microbial ID Software and Aerobe Library (Version 3.0) from Microbial ID Inc. (Newark, DE) and standard bacteriological, physiological and

15

biochemical tests enumerated below.

		STRAIN	
CHARACTER		13-5S-ACN-2a	5B-MGN-2P
20	Gram Stain	Negative	Negative
	Cell Morphology	Rod	Rod
	Flagella	Lophotrichous	Lophotrichous
	Pyocyanin	Negative	Negative
	Pyoverdin	Positive	Positive
	Argininedihydrolase	Positive	Positive
25	Growth at 41°C	Negative	Negative

5	Gelatin Hydrolysis	Negative	Negative
	Denitrification	Negative	Negative
	Starch Hydrolysis	Negative	Negative
<u>Use As Sole Carbon Source</u>			
10	Butylamine	Positive	Positive
	Inositol	Positive	Negative
	Citraconate	Positive	Negative
	L-Tartrate	Negative	Positive
	Genus species	<u>Pseudomonas putida</u>	<u>Pseudomonas putida</u>
15			
		<u>STRAIN</u>	
<u>Character</u>		<u>3L-G-1-5-1a</u>	<u>3L-A-1-5-1a-1</u>
20	Gram Strain	Negative	Negative
	Cell Morphology	Rod	Coccoid Rod
	Oxidase	Positive	Positive
	Growth on Citrate	Positive	Positive
	Urea Hydrolysis	Positive	Negative
25	Aerobic Oxidation of Dextrose	Positive	Negative
	Aerobic Oxidation of Xylose	Positive	Negative
	Indole Production	Negative	Negative
	Hydrogen Sulfide Production	Negative	Negative
	Nitrogen Production via Denitrification	Negative	Negative
30	Arginine Dihydroloase	Positive	Negative
	Dextrose Fermentation	Negative	Negative
	Motility	Not Tested	Negative
	Genus species	<u>Pseudomonas sp.</u>	Group 4 <u>Moraxella sp.</u>

- 35 For testing nitrile hydrolysis activity, PR basal medium with 10 g/L glucose was used to grow cell material. This medium was supplemented with 25 mM of (\pm)-2-methylglutaronitrile (5B-MGN-2P,

5 3L-G-1-5-1a) or 25 mM of 1,4-dicyanobutane (3L-A-1-5-1a-1, 13-5S-ACN-
2a) or 25 mM of acetamide (all strains). *P. putida* 5B-MGN-2p was also
grown in the absence of a nitrile or amide inducer with 25 mM of NH_4Cl
or $(\text{NH}_4)_2\text{SO}_4$ replacing the nitrile or amide. A 10 mL volume of
10 complete medium was inoculated with 0.1 mL of frozen stock culture (all
strains). Following overnight growth at room temperature (22-25°C) on a
shaker at 250 rpm, the 10 mL inoculum was added to 990 mL of fresh
medium in a 2-L flask. The cells were grown overnight at room
temperature with stirring at a rate high enough to cause bubble
formation in the medium. Cells were harvested by centrifugation,
15 washed once with 0.85% saline and the concentrated cell paste was
immediately placed in a -70°C freezer for storage. Thawed cell pastes
containing approximately 80% water were used in all nitrile hydrolysis
bioconversions.

The above stereospecific nitrile-hydrolyzing microorganisms were
20 representative strains from a collection of microorganisms isolated via
enrichment cultures described above. The stereospecific and
stereoselective activities of nitrile-hydrolyzing microorganisms isolated
from enrichment experiments are shown in Table 1.

25

30

35

TABLE 1
STEREOSPECIFIC/STEREOSELECTIVE HYDROLYSIS WITH SOIL ENRICHMENT ISOLATES

Microorganism ^a	Strain	Enrichment Nitrile ^b	CPIN		NPCN ^f		IBCN	
			R/Sc	Ed	R/Sc	Ed	R/Sc	Ed
<u>P. putida</u>	13-5S-ACN-2a	ACN	90/10	>10	0/100	<7	10/90	>10
<u>P. putida</u>	5B-MGN-2P	MGN	77/33	>10	43/57	>7	30/70	>10
<u>Pseudomonas</u> sp.	20-5-MGN-1P	MGN	No bioconversion		0/100	<7	No Bioconversion	
<u>Moraxella</u> sp.	3L-A-1-5-1a-1	MGN	58/42	>10	50/50	<7	44/56	>10
<u>Pseudomonas</u> sp.	3L-B-2-6-1P	ACN	No Bioconversion		59/41	<7	No Bioconversion	
<u>Pseudomonas</u> sp.	3L-G-2-5-1a	ACN	55/45	>10	0/100	<7	50/50	>10
<u>Pseudomonas</u> sp.	20-5-SBN-1a	SBN	No Bioconversion		No Bioconversion		No Bioconversion	
Not Classified	3L-G-1-2-1a	MGN	No Bioconversion		39/61	>7	No Bioconversion	
<u>Pseudomonas</u> sp.	3L-G-1-5-1a	MGN	No Bioconversion		83/17	10	No Bioconversion	
<u>Pseudomonas</u> sp.	5A-MGN-1P	MGN	No Bioconversion		N ^{Te}	N ^{Te}	No Bioconversion	
<u>Pseudomonas</u> sp.	5B-ACN-1P	ACN	58/42	>10	47/53	>10	49/51	>10
<u>Pseudomonas</u> sp.	2G-8-5-1	4CP	No Bioconversion		92/8	>10	No Bioconversion	
<u>P. auerofaciens</u>	MOB C2-1	PBN	69/31	>10	19/81	>10	0/100	>10
<u>S. liquefaciens</u>	MOB IM/N3	PPA	54/48	>10	21/79	<7	No Bioconversion	
<u>Pseudomonas</u> sp.	2G-8-5-2	4CP	No Bioconversion		68/32	<7	No Bioconversion	
<u>Pseudomonas</u>	2D-11-5-1	NAN	65/35	>10	44/56	>10	0/100	>10
<u>P. putida</u> ^g	2D-11-5-1b	NAN	69/31	>10	17/83	<7	0/100	>10

Microorganism ^a	Strain	Enrichment Nitrile ^b	CPIN		NPCN ^f		IBCN	
			R/Sc	Ed	R/S	Ed	R/Sc	Ed
<i>Pseudomonas</i> sp. ^g	2D-11-5-1c	NAN	No Bioconversion		100/0	8.8	No Bioconversion	
<i>Pseudomonas</i> sp.	20-5-SBN-1b	SBN	No Bioconversion		NT ^e	<7	10/90	>10

^a Strain identification by fatty acid analysis as described in text.

^b ACN = acetonitrile; MGN = 2-methylglutaronitrile; SBN = S-2-methylbutyronitrile; 4CP = 4-cyanophenol;

NAN = 1-naphthoacetonitrile; PBN = phenylbutyronitrile; PPA = propionamide.

^c Ratio of R-enantiomer to S-enantiomer remaining after 48-64 h incubation at 28°C; determined by chiral HPLC.

^d E = Enantiomer ratio as defined in text. Determined by reverse-phase HPLC and chiral HPLC.

^e NT = not tested.

^f Data corrected for trace of R,S-NPAm present in substrate.

^g 2D-11-5-1b and 2D-11-5-1c derived from 2D-11-5-1.

5 Microorganisms tend to undergo mutation. Thus, the bacteria, even if they are mutants of a competent strain listed above, can be used in the process according to the instant invention as long as its culture produces a stereospecific nitrile hydratase. Table 1, taken together with the disclosure presented herein, will enable one skilled in the art with a
10 minimum of experimentation to choose additional strains of Pseudomonas, Moraxella, and Serratia (and other genera as well) for converting all the nitrile starting reactants to their corresponding amides/acids.

Acid Hydrolysis of Chiral Amide to Chiral Acid

15 In the present invention, mineral acid can be used to hydrolyze the R- or S-amide derived from the R,S nitrile to the R- or S-acid. Interestingly, chiral cyanohydrins are hydrolyzed to the corresponding chiral hydroxy acids with concentrated mineral acid; see Effenberger, et al., Tetrahedron Letters, 1990, 31(9):1249-1252 and references cited
20 therein. However, we have found that 2-aryl-2-alkyl acetonitriles are not hydrolyzed by mineral acid under conditions where the corresponding chiral amides are hydrolyzed to the chiral acids. The chiral acid can be easily separated from the undesired nitrile as described below.

In addition, a chiral amide can be hydrolyzed by an amidase
25 enzyme such as the Brevibacterium and Corynebacterium strains described in EPA 326,482. This reaction does not require a stereospecific amidase and, therefore, any amidase which hydrolyzes racemic 2-aryl-alkylamides can be employed.

Step iii

Racemization of Chiral Nitriles

30 The combination of stereospecific microbial nitrile hydrolysis and mineral acid or amidase hydrolysis of amides yields a mixture of desired chiral acid and undesired chiral nitrile. Following separation of the undesired nitrile from the desired acid, e.g., by base neutralization and
35 solvent extraction of the nitrile, recycling of the R- or S- nitrile requires racemization. We have found that chiral nitriles (in which R² is H) can be converted to racemic nitriles using a strongly basic ion exchange resin

- 5 such as Amberlite® IRA-400 (OH) resin, Amberlyst® A-26, or Dowex®
1X8 resin (after exchange with hydroxide ion) in an organic solvent. This
procedure results in high racemic nitrile yields with no substantial
hydrolysis of the nitrile to the corresponding amide or acid. The resulting
racemic nitrile can be hydrolyzed to the corresponding R- or S-acid under
10 the conditions described previously.

Analytical Procedures

- Nitriles and amide and acid products derived via microbial or
mineral acid hydrolysis are measured by reverse-phase HPLC. Detection
is by ultra-violet light absorbtion. A Du Pont Zorbax® C18 column
15 employing a mobile phase of 70-75% methanol and 25-30% H₂O acidified
with 0.1% H₃PO₄ or 67% acetonitrile (ACN) and 33% H₂O acidified with
0.1% H₃PO₄ is used. Chromatographic identity and quantitation of
nitriles and their resulting amide and acid products can be determined by
comparison with authentic standards.

- 20 Chiral HPLC for the separation of enantiomers can be carried out
with an α_1 -acid glycoprotein column obtained from Chromtech (Sweden).
The mobile phases for separation of various enantiomers is summarized
below.

25 Chiral HPLC Separation of Nitrile, Amide and Acid Enantiomers

<u>Enantiomers</u>	<u>Mobile Phase</u>
CPIN, CPIAm, CPIA	95% 0.01 M Phosphate Buffer (pH 6.0):5% Ethanol
NPCN, NPAm, NPAC	95% 0.01 M Phosphate Buffer (pH 5.6):5% Ethanol
30 IBCN, IBAm, IBAC	96% 0.02 M Phosphate Buffer (pH 5.2):4% Ethanol

Enantiomeric composition, purity and chromatographic identity of
the above nitriles, amides and acids were determined by comparison with
authentic standard enantiomers or racemic mixtures.

- 35 GC analysis of CPIN, CPIAm and CPIA was carried out on a
183 cm x 2 mm (i.d.) glass column containing 3% SP2100 on Supelco

- 5 support (120 mesh). A temperature program starting at 125°C for 5 minutes and 8°C per minute to 250°C was used.

The processes of this invention are illustrated by the following Examples.

Example 1

- 10 Step i. A 100 mg (S-CPIN, R-CPIN hydrolysis) or 200 mg (R,S-CPIN hydrolysis) sample of frozen cell paste of *P. putida* 5B-MGN-2P was added to 3 mL of phosphate buffer (100 mM, pH 7.0) at room temperature. Then, 30 to 40 μ mol of S-CPIN or R-CPIN or R,S-CPIN in 120 μ L of dimethyl sulfoxide was added. After incubation at 28°C with 15 agitation for 48 h, the reactions were acidified with 3 M H₂SO₄ to pH 3.0. Four volumes of methylene chloride were added to each sample and the suspensions were agitated for 15-30 minutes. The methylene chloride layers were removed and evaporated to dryness under a stream of nitrogen and the residues were resuspended in 3 mL of methanol. The 20 composition of the methanol solution was determined by reverse-phase HPLC and chiral HPLC and is shown in Table 2.

Table 2

S-CPIN, R-CPIN and R,S-CPIN Hydrolysis by *P. putida*, 5B-MGN-2P

25

Substrate (μ mol added)	HPLC Analysis (μ mol recovered)					
	Reverse Phase		Chiral			
	CPIN	CPIAm	S-CPIN	R-CPIN	S-CPIAm	R-CPIAm
S-CPIN (30.9)	1.8	28.2	1.8	ND ^a	28.2	ND ^a
R-CPIN (37.5)	33.7	3.8	NT ^b	NT ^b	NT ^b	NT ^b
R,S-CPIN (31.9)	20.4	11.5	4.7	15.7	11.0	0.5

^a ND = None Detected.

^b NT = Not Tested.

5

Example 2

Step i: A 50 mg sample of frozen cell paste of *P. putida* 5B-MGN-2p obtained from cultures propagated on PR glucose medium supplemented with 25 mM NH₄Cl in place of 25 mM (±)-2-methylglutaronitrile was added to 1 mL of pyrophosphate buffer (5 mM, pH 7.5) at room temperature containing 20.6 μmole of S-CPIN or R,S-CPIN. After incubation at 28°C with agitation for 24 h, the reaction was acidified with 3 M H₂SO₄ to pH 3.0. Four volumes of methylene chloride were added to each sample and the suspensions were agitated for 15-30 min. The methylene chloride layers were removed and evaporated to dryness under a stream of nitrogen and the residues were resuspended in 1 mL of methanol. The composition of the methanol solution was determined by reverse-phase HPLC and chiral HPLC and is shown in Table 3.

20

Table 3

S-CPIN, R,S-CPIN Hydrolysis by *P. putida*, 5B-MGN-2P
Propagated in the Absence of Nitrile or Amide Inducer

Substrate (μmol added)	HPLC Analysis (μmol recovered)					
	Reverse Phase		Chiral			
	CPIN	CPIAm	S-CPIN	R-CPIN	S-CPIAm	R-CPIAm
S-CPIN (20.6)	1.2	17.7	NT ^a	NT ^a	NT ^a	NT ^a
R,S-CPIN (20.6)	15.1	3.3	6.0	9.1	3.3	ND ^b

^a NT = Not Tested.

25 ^b ND = None Detected.

Example 3

Step i. A 20 mg sample of frozen cell paste of *P. putida* 5B-MGN-2P was added to 1 mL of phosphate buffer (0.3 mM, pH 7.2) containing MgCl₂•6H₂O (2 mM) at room temperature. Then 0.95 μmol of R,S-NPCN in 40 μL of dimethyl sulfoxide was added. After incubation at 28°C with

30

- 5 Four volumes of methylene chloride were added and the suspension was agitated for 30 min. The methylene chloride layer was removed and evaporated to dryness under a stream of nitrogen and the residue was resuspended in 1 mL of methanol. The composition of the extracted supernatant was determined by reverse-phase HPLC and chiral HPLC
- 10 and is shown in Table 4.

TABLE 4
R,S-NPCN Hydrolysis by *P. putida* 5B-MGN-2P

Substrate (mmol added)	HPLC Analysis (μ mol recovered)							
	Reverse Phase ^b			Chiral ^b				
	NPCN	NPAm	NPAC	S-NPCN	R-NPCN	S-NPAm	R-NPAm	S-NPAC
R,S-NPCN (0.95)	0.5	0.22	0.03	0.28	0.22	ND ^a	0.22	0.03

15 ^a ND = None Detected.

^b Data corrected for trace of R,S-NPAm present in substrate.

Example 4

- 20 Step i. A 40 mg sample of frozen cell paste of *P. putida* 5B-MGN-2P was added to 1 mL of phosphate buffer (100 mM, pH 7.0) at room temperature. Then 10.7 μ mol of R,S-IBCN in 40 μ L of dimethylsulfoxide was added. After incubation at 28°C with agitation for 48 h, the reaction was acidified with 3 M H₂SO₄ to pH 3.0. Four volumes of methylene chloride were added and the suspension was agitated for
- 25 15-30 min. The methylene chloride layer was removed and evaporated to dryness under a stream of nitrogen and the residue was resuspended in 1 mL of methanol. The composition of the extracted supernatant is determined by reverse phase HPLC and chiral HPLC and is shown in Table 5.

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TABLE 5**R,S-IBCN Hydrolysis by *P. putida* 5B-MGN-2P**

Substrate (μ mol added)	HPLC Analysis (μ mol recovered)					
	Reverse Phase		Chiral			
	IBCN	IBAm	S-IBCN	R-IBCN	S-IBAm	R-IBAm
R,S-IBCN (10.7)	7.9 ^a	2.8	5.5 ^a	2.4 ^a	ND ^b	2.8

- 10 ^a Estimated value calculated by subtracting μ mol IBAm recovered from μ mol IBCN added.
 ^b ND = None Detected.

Example 5

- 15 Step i. A 50 mg sample of frozen cell paste of *Moraxella* sp. 3L-A-1-5-1a-1 was added to 1 mL of phosphate buffer (100 mM, pH 7.0) at room temperature. Then 10.3 μ mol of S-CPIN, R-CPIN or R,S-CPIN in 40 μ L of dimethyl sulfoxide was added. After incubation at 28°C with agitation for 48 h, the reactions were acidified with 3 M H₂SO₄ to pH 3.0.
- 20 Four volumes of methylene chloride was added to each sample and the suspensions were agitated for 15-30 minutes. The methylene chloride layers were removed and evaporated to dryness under a stream of nitrogen and the residues were resuspended in 1 mL of methanol. The composition of the methanol solution was determined by reverse-phase
- 25 HPLC and chiral HPLC and is shown in Table 6.

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TABLE 6

S-CPIN, R-CPIN and R,S-CPIN Hydrolysis by Moraxella sp. 3L-A-1-5-1a-1

Substrate (μ mol added)	HPLC Analysis (μ mol recovered)					
	Reverse Phase		Chiral			
	CPIN	CPIAm	S-CPIN	R-CPIN	S-CPIAm	R-CPIAm
S-CPIN (10.3)	0.5	8.7	0.5	ND ^a	8.7	ND ^a
R-CPIN (10.3)	10.3	ND ^a	NT ^b	NT ^b	NT ^b	NT ^b
R,S-CPIN (10.3)	9.7	0.5	4.1	5.6	0.5	ND ^a

a ND = None Detected.

10 b NT = Not Tested.

Example 6

Step i. A 40 mg sample of frozen cell paste of Moraxella sp. 3L-A-1-5-1a-1 was added to 1 mL of phosphate buffer (100 mM, pH 7.0) at room temperature. In the same manner as in Example 4, 10.7 μ mol of R,S-IBCN was added. Following the same incubation and extraction protocols as in Example 4, the composition of the extracted supernatant was determined by reverse-phase and chiral HPLC. The results are shown in Table 7.

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TABLE 7

R,S-IBCN Hydrolysis by *Moraxella* sp. 3L-A-1-5-1a-1

Substrate (μ mol added)	HPLC Analysis (μ mol recovered)								
	Reverse Phase			Chiral					
	IBCN	IBAm	IBAC	S- IBCN	R- IBCN	S- IBAm	R- IBAm	S- IBAC	R- IBAC
R,S-IBCN (10.7)	9.8 ^a	0.4	0.5	5.5 ^a	4.3 ^a	ND ^b	0.4	ND ^b	0.5

- 10 ^a Estimated value calculated by subtracting μ mol amide recovered from
 μ mol IBCN added.
 ^b ND = None Detected.

Example 7

- 15 Step i. A 20 mg sample of frozen cell paste of *Pseudomonas* sp.
 3L-G-1-5-1a, was added to phosphate buffer (0.3 mM, pH 7.0) containing
 MgCl₂•6H₂O (2 mM) at room temperature. In the same manner as in
 Example 3, 0.95 μ mol of R,S-NPCN was added. Following the same
 incubation and extraction as in Example 3, the composition of the
20 extracted supernatant was determined by reverse-phase HPLC and chiral
 HPLC. The results are shown in Table 8.

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TABLE 8

R,S-NPCN Hydolysis by *Pseudomonas* sp. 3L-G-1-5-1a

Substrate (mmol added)	HPLC Analysis (μ mol recovered)							
	Reverse Phase ^b			Chiral ^b				
	NPCN	NPAm	NPAC	S-NPCN	R-NPCN	S-NPAm	R-NPAm	S-NPAC
R,S-NPCN (0.95)	0.44	0.3	0.30	0.03	0.41	ND ^a	0.03	0.30

a ND = None Detected.

b Data corrected for trace of R,S-NPAm present in substrate.

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Example 8

Step i. A 100 mg sample of frozen cell paste of *P. putida* 13-5S-ACN-2a was added to 3 mL of phosphate buffer (100 mM, pH 7.0) at room temperature. In the same manner as in Example 1, 30.9 μ mol of S-CPIN, R-CPIN or R,S-CPIN was added. Following the same incubation and extraction protocols as in Example 1, the composition of the extracted supernatants was determined by reverse-phase HPLC and chiral HPLC. The results are shown in Table 9.

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TABLE 9

S-CPIN, R-CPIN, R,S-CPIN Hydrolysis by *P. putida* 13-5S-ACN-2a

Substrate (μ mol added)	HPLC Analysis (μ mol recovered)					
	Reverse Phase		Chiral			
	CPIN	CPIAm	S-CPIN	R-CPIN	S-CPIAm	R-CPIAm
S-CPIN (30.9)	ND ^a	30.2	ND ^a	ND ^a	30.2	ND ^a
R-CPIN (30.9)	28.5	0.6	NT ^b	NT ^b	NT ^b	NT ^b
R,S-CPIN (30.9)	13.2	14.7	1.3	11.9	14.0	0.7

a ND = None Detected.

10 b NT = Not Tested.

Example 9

Step i. A 40 mg example of frozen cell paste of *P. putida* 13-5S-ACN-2a was added to phosphate buffer (100 mM, pH 7.0) at room temperature. In the same manner as in Example 4, 10.7 μ mol of R,S-IBCN was added. Following the same incubation and extraction protocols as in Example 4, the composition of the extracted supernatant was determined by reverse-phase HPLC and chiral HPLC. The results are shown in Table 10.

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Table 10

R,S-IBCN Hydrolysis by *P. putida* 13-5S-ACN-2a

Substrate (μ mol added)	HPLC Analysis (μ mol recovered)					
	Reverse Phase		Chiral			
	IBCN	IBAm	S-IBCN	R-IBCN	S-IBAm	R-IBAm
R,S-IBCN (10.7)	6.6 ^a	4.1	5.9 ^a	0.7 ^a	ND ^b	4.1

10 ^a Estimated value calculated by subtracting μ mol IBAm recovered from μ mol IBCN added.

^b ND = None Detected.

Example 10

15 Step i. A 50 mg sample of frozen cell paste of *P. putida* 2D-11-5-1b was added to 1 mL of phosphate buffer (100 mM, pH 7.0) at room temperature. In the same manner as Example 5, 10.3 μ mol of S-CPIN or R,S-CPIN was added. Following the same incubation and extraction protocols as in Example 5, the composition of the extracted supernatants was determined by reverse-phase HPLC and chiral HPLC. The results
20 are shown in Table 11.

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TABLE 11**S-CPIN, R,S-CPIN Hydrolysis by *P. putida* 2D-11-5-1b**

Substrate (μ mol added)	HPLC Analysis (μ mol recovered)					
	Reverse Phase		Chiral			
	CPIN	CPIAm	S-CPIN	R-CPIN	S-CPIAm	R-CPIAm
S-CPIN (10.3)	ND	10.0	ND	ND	10.0	ND
R,S-CPIN (10.3)	8.5	3.0	2.6	5.9	3.0	ND

ND = None Detected.

- 10 Apparent excess recovery of CPIN was most likely due to experimental error.

Example 11

- 15 Step i. A 50 mg sample of frozen cell paste of *P. putida* 2D-11-5-1b was added to 2 mL of phosphate buffer (100 mM, pH 7.0) at room temperature. In the same manner as in Example 4, 10.7 μ mol of R,S-IBCN was added. Following the same incubation and extraction protocols as in Example 4, the composition of the extracted supernatant was determined by reverse-phase HPLC and chiral HPLC. The results
- 20 are shown in Table 12.

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TABLE 12

R,S-IBCN Hydrolysis by *P. putida* 2D-11-5-1b

Substrate (μ mol added)	HPLC Analysis (μ mol recovered)					
	Reverse Phase		Chiral			
	IBCN	IBAm	S-IBCN	R-IBCN	S-IBAm	R-IBAm
R,S-IBCN (10.7)	7.1 ^a	3.6	5.0 ^a	2.1 ^a	0.4	3.2

^a Estimated value calculated by subtracting μ mol IBAm recovered from μ mol IBCN added.

10

Example 12

Step i. A 50 mg sample of frozen cell paste of *S. liquefaciens* MOB IM/N3 was added to 1 mL of phosphate buffer (100 mM, pH 7.0) at room temperature. In the same manner as Example 5, 10.3 μ mol of S-CPIN, R-CPIN or R,S-CPIN was added. Following the same incubation and extraction protocols as in Example 5, the composition of the extracted supernatants was determined by reverse-phase HPLC and chiral HPLC. The results are shown in Table 13.

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TABLE 13

S-CPIN, R-CPIN Hydrolysis by *S. liquefaciens* MOB IM/N3

HPLC Analysis (μ mol recovered)						
Substrate (μ mol added)	Reverse Phase		Chiral			
	CPIN	CPIAm	S-CPIN	R-CPIN	S-CPIAm	R-CPIAm
S-CPIN (10.3)	0.8	8.2	NT ^a	NT ^a	NT ^a	NT ^a
R-CPIN (10.3)	9.5	<0.1	NT ^a	NT ^a	NT ^a	NT ^a
R,S-CPIN (10.3)	8.6	1.2	4.0	4.6	1.2	ND ^b

a NT = Not Tested.

b ND = None Detected.

10

Example 13

Step i. A 50 mg sample of frozen cell paste of *P. aureofaciens* MOB C2-1 was added to 1 mL of phosphate buffer (100 mM, pH 7.0) at room temperature. In the same manner as Example 5, 10.3 μ mol of S-CPIN, R-CPIN or R,S-CPIN was added. Following the same incubation and extraction protocols as in Example 5, the composition of the extracted supernatants was determined by reverse-phase HPLC and chiral HPLC. The results are shown in Table 14.

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TABLE 14

S-CPIN, R-CPIN, R,S-CPIN Hydrolysis by *P. aureofaciens* MOB C2-1

Substrate (μ mol added)	HPLC Analysis (μ mol recovered)					
	Reverse Phase		Chiral			
	CPIN	CPIAm	S-CPIN	R-CPIN	S-CPIAm	R-CPIAm
S-CPIN (10.3)	ND ^a	8.4	ND ^a	ND ^a	8.4	ND ^a
R,CPIN (10.3)	9.0	<1.0	NT ^b	NT ^b	NT ^b	NT ^b
R,S-CPIN (10.3)	8.4	1.0	2.6	5.8	1.0	ND ^a

a ND = None Detected.

b NT = Not Tested.

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Example 14

Step i. A 50 mg sample of frozen cell paste of *P. aureofaciens* MOB C2-1 was added to 1 mL of phosphate buffer (100 mM, pH 7.0) at room temperature. In the same manner as in Example 4, 10.7 μ mol of R,S-IBCN was added. Following the same incubation and extraction protocols as in Example 4, the composition of the extracted supernatant was determined by reverse-phase HPLC and chiral HPLC. The results are shown in Table 15.

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TABLE 15

R,S-IBCN Hydrolysis by *P. aureofaciens* MOB C2-1

Substrate (μ mol added)	HPLC Analysis (μ mol recovered)					
	Reverse Phase		Chiral			
	IBCN	IBAm	S-IBCN	R-IBCN	S-IBAm	R-IBAm
R,S-IBCN (10.7)	8.3 ^a	2.4	5.4 ^a	2.9 ^a	ND ^b	2.4

10 ^a Estimated value calculated by subtracting μ mol IBAm recovered from
 μ mol IBCN added.

^b None Detected.

Example 15

15 Step i. Approximately 20 mg of frozen cell paste of
 Pseudomonas sp., 2G-8-5-1a, was added to 1 mL of phosphate buffer (0.1
 M, pH 7.2) at room temperature. Then approximately 1 μ mol of
 R,S-NPCN in 40 μ L of dimethyl sulfoxide was added. After incubation at
 28°C with agitation for 48 h, the reaction was acidified to pH 3.0 with 3
 M H₂SO₄. Four volumes of methylene chloride were added and the
20 suspension was agitated for 30 min. The methylene chloride layer was
 removed and evaporated to dryness under a stream of nitrogen. The
 residue was redissolved in 1 mL of methanol. The composition of the
 extracted supernatant was determined by reverse-phase HPLC and chiral
 HPLC as described elsewhere. The results are shown in Table 16.

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TABLE 16
R,S-NPCN hydrolysis by *Pseudomonas* sp., 2G-8-5-1a

HPLC Analysis (μ mol recovered)

	Reverse Phase ^b			Chiral ^b					
	NPCN	NPAm	NPac	S-NPCN	R-NPCN	S-NPAm	R-NPAm	S-NPac	R-NPac
Substrate (μmol added)									
R,S-NPCN (0.95)	0.52	0.04	0.36	ND ^a	0.52	ND	0.04	0.36	ND

^a ND = None Detected.

^b Data corrected for trace of R,S-NPAm present in substrate.

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TABLE 17
R,S-NPCN hydrolysis by *Pseudomonas* sp., 2D-11-5-1c

HPLC Analysis (μmol recovered)

Substrate (μmol added)	Reverse Phase ^b			Chiral ^b					
	NPCN	NPAm	NPac	S-NPCN	R-NPCN	S-NPAm	R-NPAm	S-NPac	R-NPac
R,S-NPCN (0.95)	0.66	0.09	0.40	ND ^a	0.66	0.04	0.05	0.4	ND

^a ND = None Detected.

^b Data corrected for trace of R,S-NPAm present in substrate.

5

Example 17

Step i. Approximately 2 mg of frozen cell paste of *P. aureofaciens*, MOB C2-1, was added to 1 mL of phosphate buffer (0.1 M, pH 7.2) at room temperature. Then approximately 1 μ mol of R,S-NPCN in 40 μ L of dimethyl sulfoxide was added. After incubation at 28°C with agitation for 48 h, the reaction was acidified to pH 3.0 with 3 M H₂SO₄. Four volumes of methylene chloride were added and the suspension was agitated for 30 min. The methylene chloride layer was removed and evaporated to dryness under a stream of nitrogen. The residue was redissolved in 1 mL of methanol. The composition of the extracted supernatant was determined by reverse-phase HPLC and chiral HPLC as described elsewhere. The results are shown in Table 18.

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TABLE 18
R,S-NPCN hydrolysis by *P. aureofaciens*, MOB C2-1

HPLC Analysis (μ mol recovered)

Substrate (μ mol added)	Reverse Phase ^b			Chiral ^b			
	NPCN	NPAm	NPAc	S-NPCN	R-NPCN	S-NPAm	R-NPAm
R,S-NPCN (0.95)	0.66	0.17	ND	0.53	0.13	ND	0.17
						ND	ND

^a ND = None Detected.

^b Data corrected for trace of R,S-NPAm present in substrate.

5

Example 18

Step ii. A suspension of 1.00 g of S-CPIAm in 16 mL of aqueous hydrochloric acid (18%) was stirred and heated to reflux. As the suspension was heated, the solid dissolved. After 16 h, the reaction mixture was cooled. The solid which precipitated and solidified around the stirrer was extracted with methylene chloride. Evaporation of the extract left 0.98 g of colorless solid which was analyzed by a combination of GC and HPLC. It was shown by GC to be mainly CPIA (92.3 area percent) with the remainder being unchanged amide. The configuration of the acid was established, by chiral HPLC as being the S-enantiomer (at least 98.2%), with only a trace of the racemized R-enantiomer.

Example 19

Step ii. The reaction was repeated as in Example 18 using 1.02 g of S-CPIAm and 15 mL of concentrated hydrochloric acid. After approximately 16 h at reflux, the reaction mixture was cooled and the precipitated solid was collected by filtration and air dried. There was recovered 0.96 g of colorless solid which was characterized by GC/mass spectrometry and by HPLC. The major component was identified as CPIA (96%) with about 4% of unchanged amide. Chiral HPLC showed that the acid was 96.6% of the S-enantiomer and 3.4% of the R-enantiomer.

Example 20

Step iii. One g of wet Amberlite® IRA-400 (OH⁻ form) was treated with 10 mL of 5% NaOH for 10 min with stirring, filtered and washed with distilled water until the washings were neutral. The solid was suspended in 25 mL of absolute ethanol and 1.06 g of R-CPIN was added. This was stirred and heated to reflux for 64 h. After removal of the resin by filtration, the filtrate was cooled and rotary-evaporated to leave 1.01 g of colorless oil. Chiral HPLC analysis showed the oil to be a 50/50 mixture of R- and S-CPIN.

5 A method that shows the relative stability of R,S alkyl nitriles such
as CPIN and their lack of conversion to the corresponding acids under
relatively strong reaction conditions is as follows. A suspension of 9.70 g
of R,S-CPIN in 100 mL of concentrated hydrochloric acid was heated to
10 reflux for 16 h. The reaction mixture was cooled and extracted three
times with methylene chloride. The combined extracts were washed with
water and dried over anhydrous magnesium sulfate. Removal of the
solvent left a colorless oil which was characterized by GC. There was a
single main component (over 90%) with the same retention time as
15 authentic starting nitrile. There was no evidence for the corresponding
acid which would be produced by hydrolysis.

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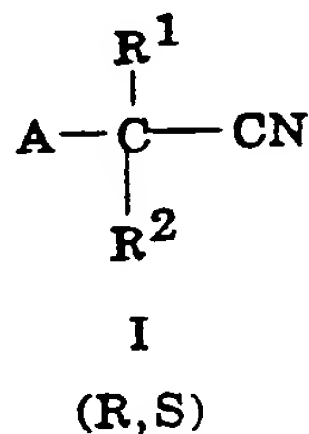
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CLAIMS

What is claimed is:

1. A method for converting a nitrile of the formula

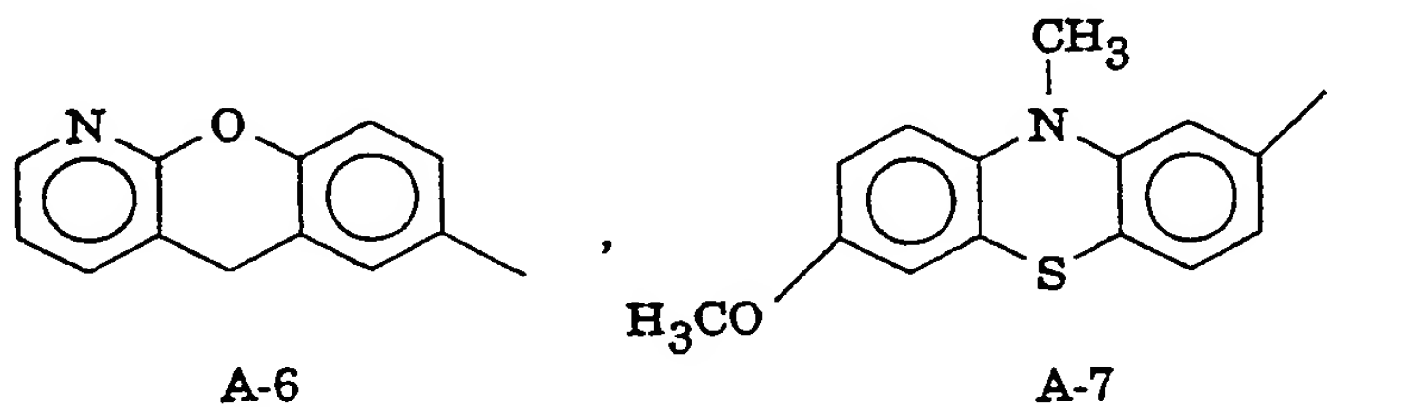
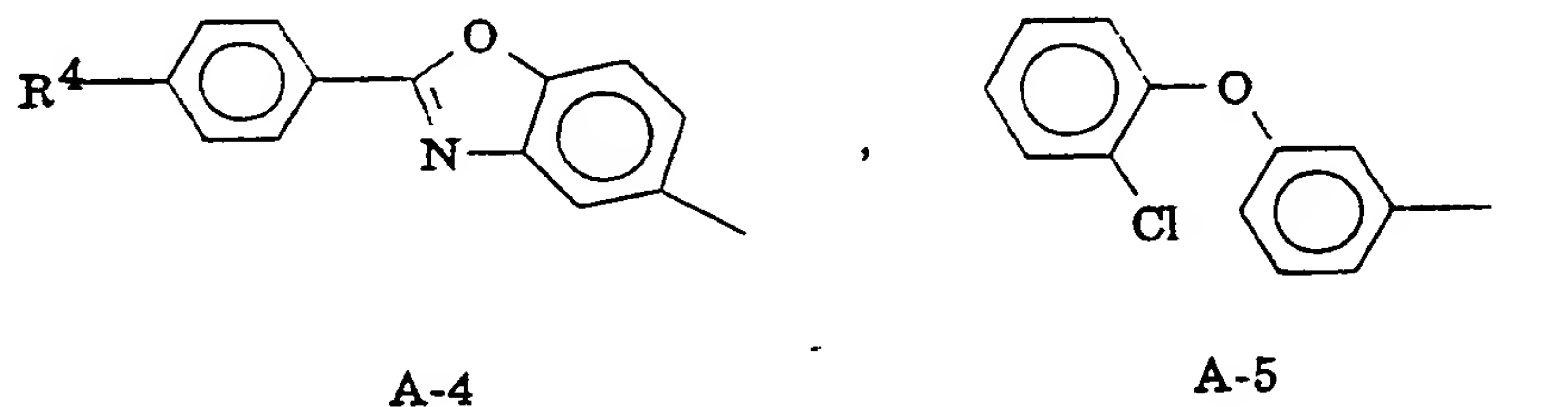
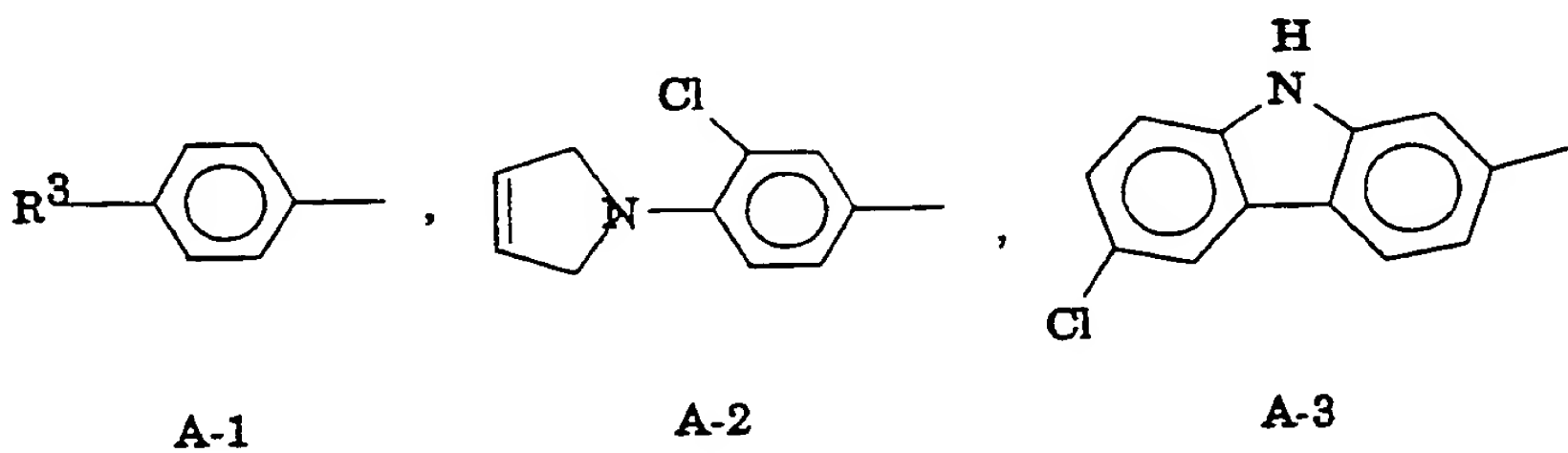
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wherein:

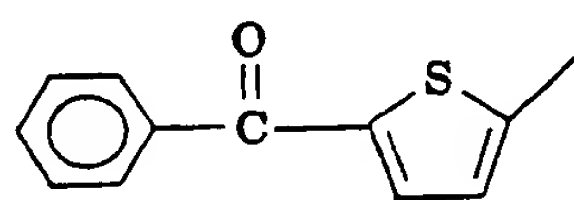
A is selected from the group consisting of:

15

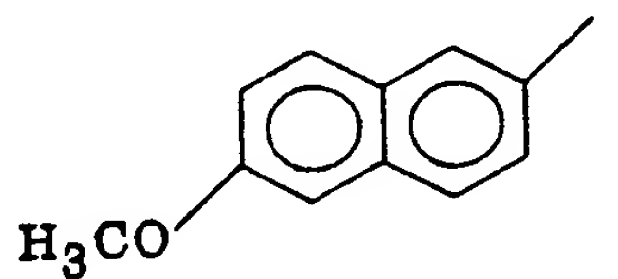


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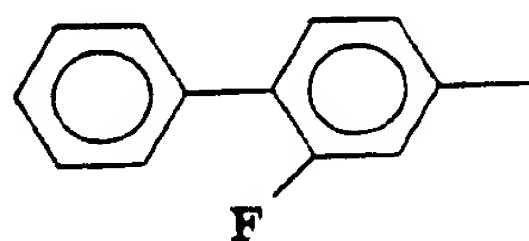
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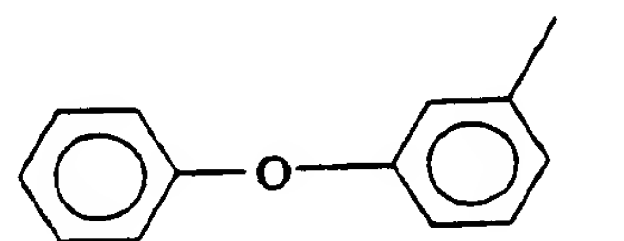


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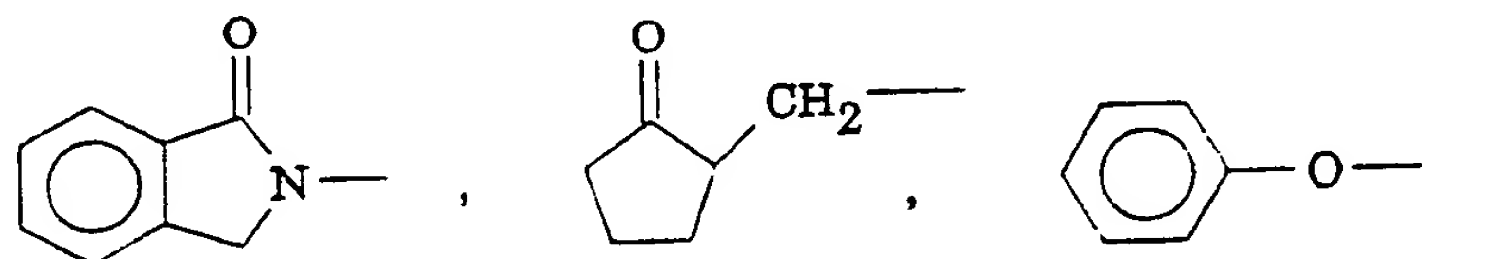
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and

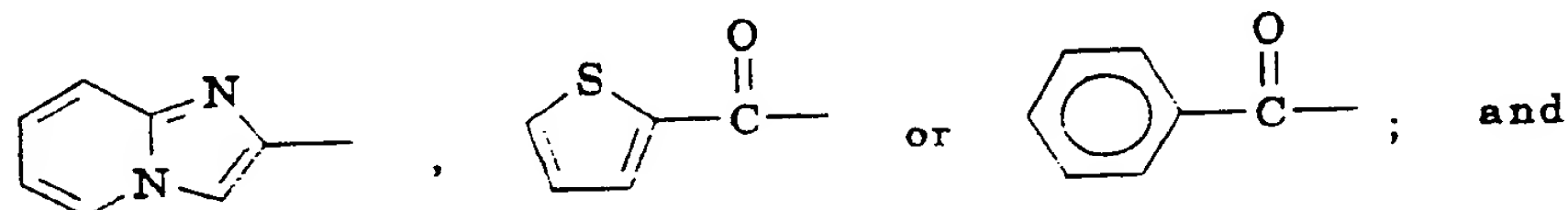


A-11

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 R^1 is C_1 - C_4 alkyl; R^2 is H or OH; R^3 is H, Cl, OCF_2H , $(CH_3)_2CHCH_2$, $H_2C=C(CH_3)CH_2NH$,

15

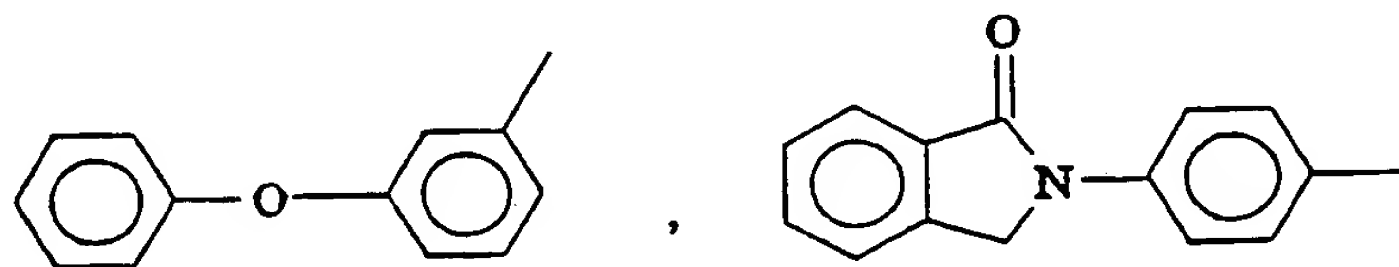
 R^4 is Cl or F;

to the corresponding amide comprising contacting said nitrile with a
 20 biological material that stereospecifically converts the racemic nitrile to
 the corresponding enantiomeric R- or S-amide.

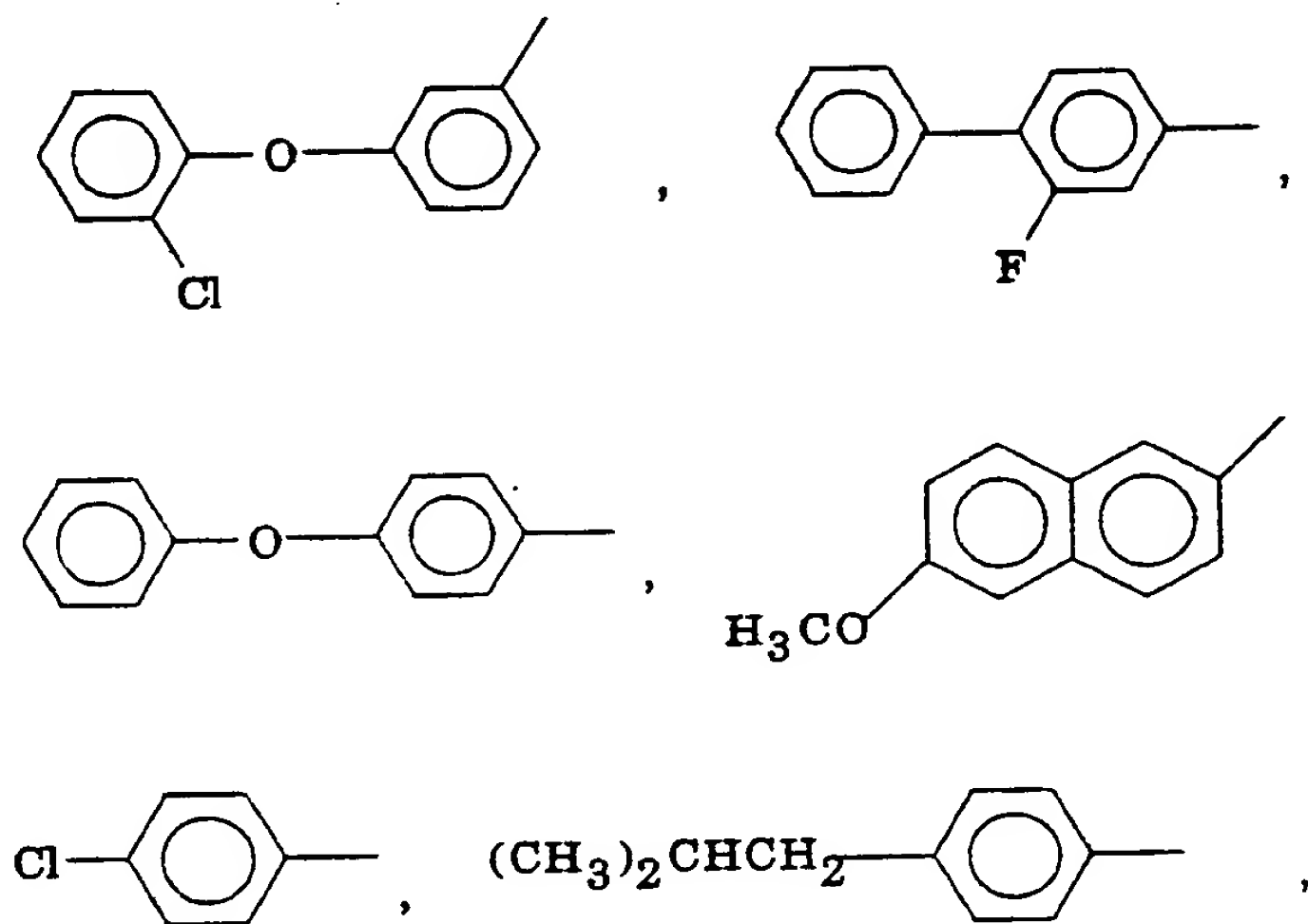
2. A method according to Claim 1 wherein A is selected from
 the group

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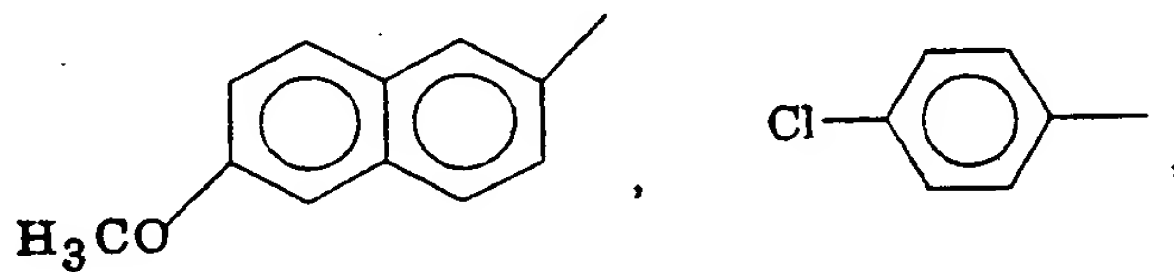


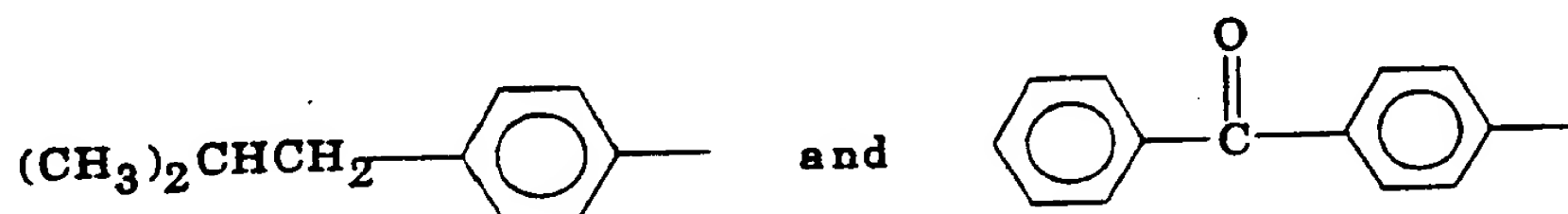
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R^1 is selected from CH_3 and $CH(CH_3)_2$.

3. A method according to Claim 2 wherein A is selected from the group

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4. A method according to Claim 3 wherein the nitrile is selected from the group (2-(4-chlorophenyl)-3-methylbutyronitrile, 2-(4-isobutylphenyl)propionitrile and 2-(6-methoxy-2-naphthyl)-propionitrile.

10

5. A method according to Claim 4 wherein the nitrile is selected from the group 2-(4-chlorophenyl)-3-methylbutyronitrile and 2-(6-methoxy-2-naphthyl)propionitrile.

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6. A method according to Claim 1 wherein the biological material is located in or derived from the group Pseudomonas, Moraxella and Serratia.

20

7. A method according to Claim 6 wherein the biological material is located in or derived from Pseudomonas putida, Moraxella sp. and Serratia liquefaciens.

25

8. A method according to Claim 7 wherein the biological material is located in or derived from Pseudomonas putida.

9. A method according to Claim 7 wherein the biological material is located in or derived from Moraxella sp.

30

10. A method according to Claim 7 wherein the biological material is located in or derived from Serratia liquefaciens.

5 11. A method according to Claim 1 comprising the additional
step of hydrolyzing the enantiomeric amide to the corresponding
2-alkanoic acid.

10 12. A method according to Claim 11 employing a strong mineral
acid to hydrolyze the amide to the acid.

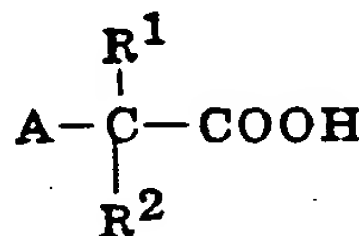
13. A method according to Claim 11 employing a biological
material to hydrolyze the amide to the acid.

15 14. A method according to Claim 13 wherein the biological
material is located in or derived from a strain selected from
Brevibacterium, Corynebacterium, Pseudomonas, Serratia and
Moraxella.

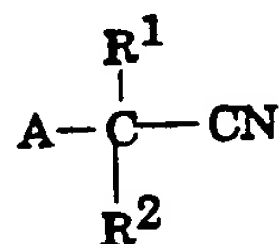
20 15. A continuous method for making an enantiomeric 2-alkanoic
acid according to Claim 11 in which R² is H, comprising continuously
removing the acid, racemizing the enantiomeric nitrile by-product of the
nitrile-to-acid reaction by contacting it with a strongly basic ion-exchange
resin, and recycling the racemic nitrile.

25 16. A method according to Claim 15 wherein the ion exchange
resin is a cross-linked copolymer containing a quaternary ammonium
hydroxide functionality.

30 17. A method for preparing an acid of the formula:

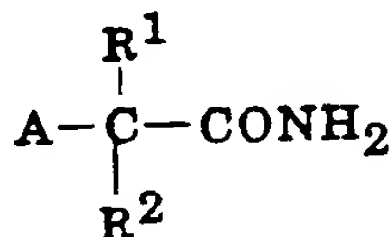


5 by hydrolyzing an admixture of the R- or S-enantiomer of



and an R- or S-amide of the formula

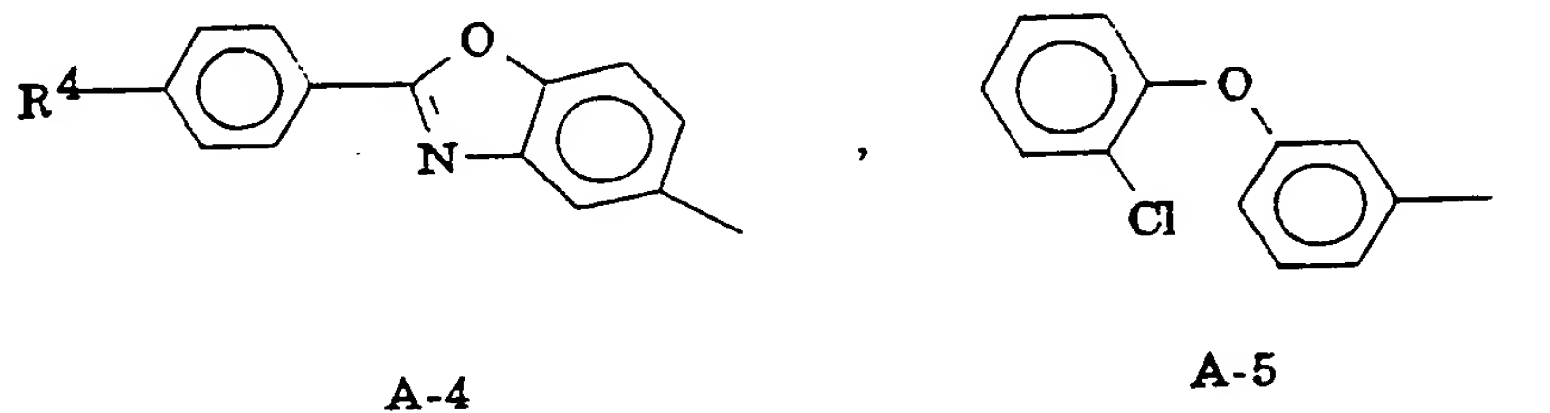
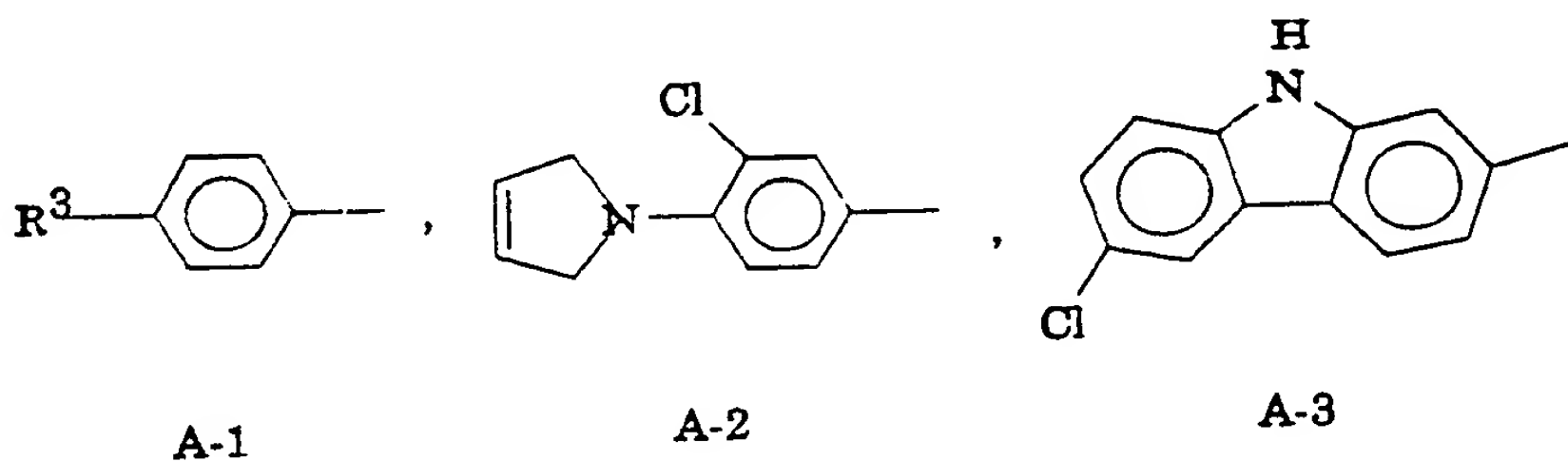
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in the presence of strong mineral acid or biological materials,

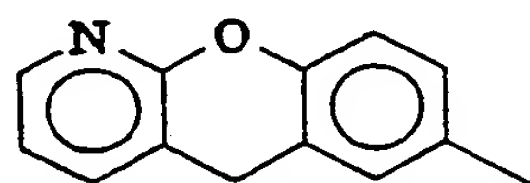
15 wherein:

A is selected from the group consisting of:

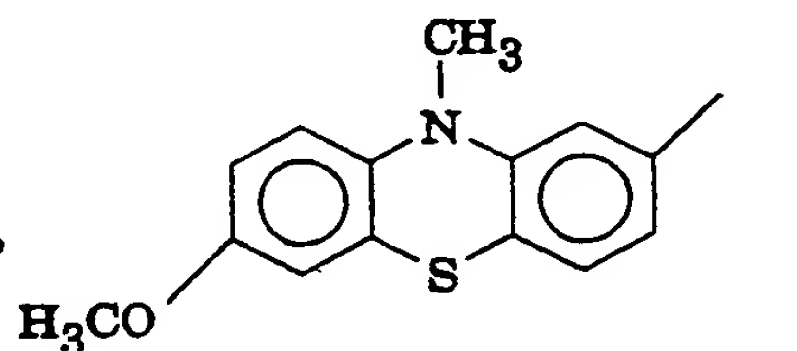


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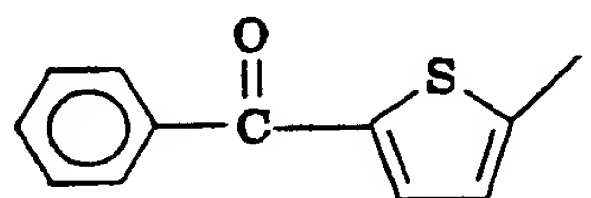
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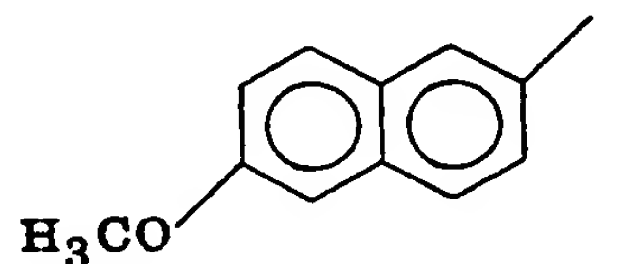
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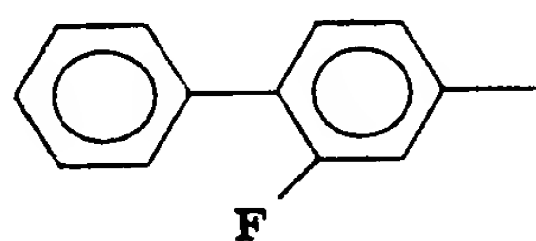
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A-8

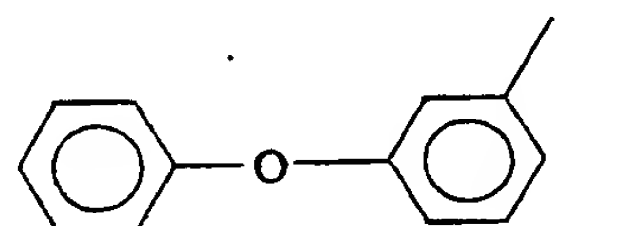


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and

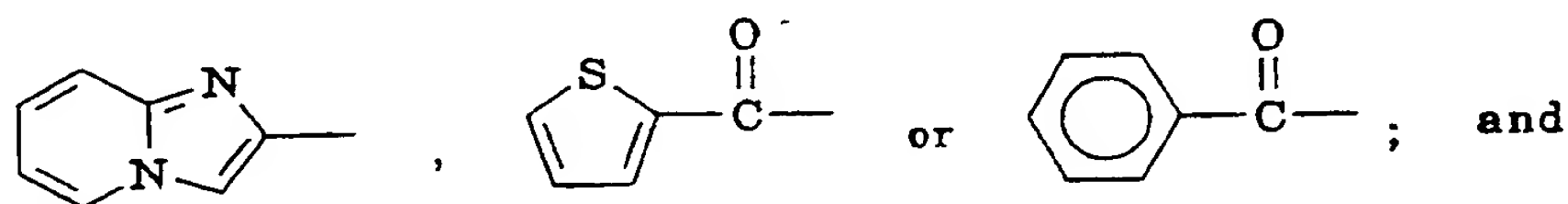
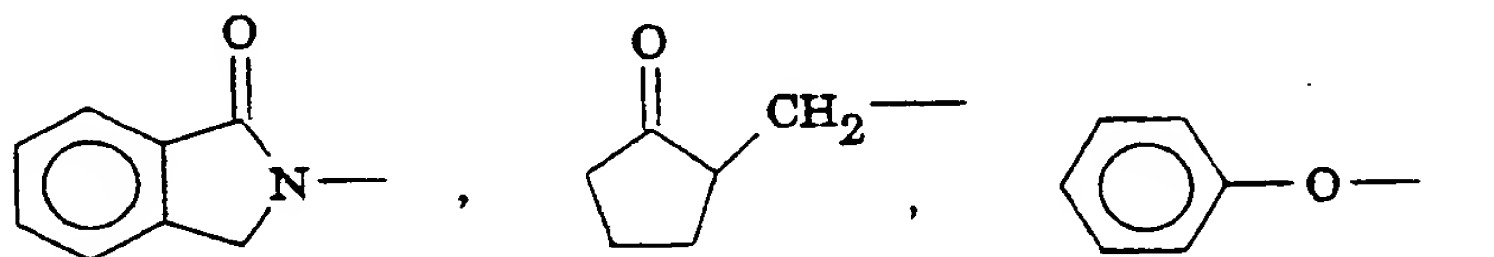


A-11

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 R^1 is C_1 - C_4 alkyl; R^2 is H or OH; R^3 is H, Cl, OCF_2H , $(CH_3)_2CHCH_2$, $H_2C=C(CH_3)CH_2NH$,

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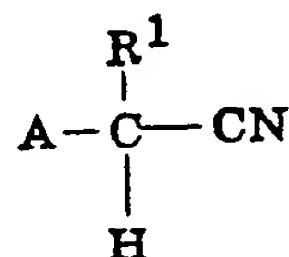
and

 R^4 is Cl or F.

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5 18. A method according to Claim 17 wherein the mineral acid is HCl and the biological material is located in or derived from a strain selected from Brevibacterium, Corynebacterium, Pseudomonas, Serratia and Moraxella.

10 19. A method for racemizing an enantiomeric R- or S-nitrile of the formula



15 comprising contacting said nitrile with a strongly basic ion-exchange resin.

20 20. A method according to Claim 19 wherein the ion-exchange resin is a cross-linked copolymer containing a quaternary ammonium hydroxide functionality.

25 21. A method according to any one of Claims 11 to 18 wherein the amide is selected from the group 2-(4-chlorophenyl)-3-methylbutyramide, 2-(4-isobutylphenyl)propionamide and 2-(6-methoxy-2-naphthyl)propionamide.

30 22. A method according to any one of Claims 19 to 20 wherein the nitrile is selected from the group 2-(4-chlorophenyl)-3-methylbutyronitrile, 2-(4-isobutylphenyl)propionitrile and 2-(6-methoxy-2-naphthyl)propionitrile.

 23. A biological material located in or derived from Pseudomonas sp. 3L-G-1-5-1a, Pseudomonas sp. 2G-8-5-1a, P. putida 5B-MGN-2p and P. aureofaciens MOB C2-1, or a variant or mutant

5 thereof, which material stereospecifically converts a racemic nitrile to the corresponding enantiomeric R- or S-amide.

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 91/06482

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ⁶		
According to International Patent Classification (IPC) or to both National Classification and IPC		
Int.Cl. 5	C12P41/00; C12P17/18;	C12P13/02; /(C12P41/00; C12R1/38)
C12P17/00; C12P17/10		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
Int.Cl. 5	C12P	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸		
III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹		
Category ¹⁰	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
X Y	EP,A,0 330 529 (RHONE POULENC) 30 August 1989 see page 3; claims ---	1, 14 1-4, 15, 17
X Y	EP,A,0 356 912 (IDEMITSU KOSAN) 7 March 1990 see page 2 see page 3; claims ---	1, 2, 4, 6, 7, 14 3
X	EP,A,0 326 482 (RHONE POULENC) 2 August 1989 see claims ---	1, 14
Y	EP,A,0 348 901 (ASAHI KASEI KOGYO KABUSHIKI) 3 January 1990 see page 4 - page 11 see page 20; claims ---	1-4, 15, 17
<p>¹⁰ Special categories of cited documents: ¹⁰</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&" document member of the same patent family</p>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search 30 JANUARY 1992		Date of Mailing of this International Search Report 12 FEB 1992
International Searching Authority EUROPEAN PATENT OFFICE		Signature of Authorized Officer DELANGHE L. L. M. 

**ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO.**

US 9106482
SA 52504

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 30/01/92

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		CN-A-	1035847	27-09-89
		JP-A-	1309695	14-12-89

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		CN-A-	1035846	27-09-89
		JP-A-	1240199	25-09-89
		US-A-	5034329	23-07-91

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